International Journal of Innovative Research and Advanced Studies (IJIRAS)
Volume 6 Issue 12, December 2019

ISSN: 2394-4404

Synthesis, Characterization And Antimicrobial Activity Of 2-Aminopyridine-Cephalexin Schiff Base And Its Mn(II), Co(II) AND Cu(II) Complexes

D Ibrahim

N .P. Ndahi

Department of Chemistry, Faculty of Science, University of Maiduguri, Borno State Nigeria

B. B Paul

Department of Veterinary Medicine, University of Maiduguri, Borno State- Nigeria

O. A. Wafudu Handy

Department of Chemistry, Faculty of Science, UniversitiTeknologi, Malaysia, Skudai, Johor, Malaysia

Abstract: ***Three transition metal complexes of Mn(II), Co(II), and Cu(II) salts were prepared from novel Schiff base ligand, ceph-2-ampy(HL) which was synthesized from the interaction of cephalexin and 2-aminopyridine. The Schiff base ligand and all its corresponding complexes are colored, and are found to be very stable at room temperature, soluble in organic solvents such as methanol, ethanol, dimethylsulfoxide (DMSO) and dimethylformamide (DMF). The percentage yield of the ligand and its complexes ranges from 63-66%, while the melting points range from 146-195^{o}C.The complexes were structurally characterized using Fourier transform infrared (FT-IR), UV-visible (UV-Vis), metal analysis, molar conductivity and nuclear magnetic resonance (^{1}H-NMR) of the ligand. From the spectral analyses, the molar conductivity measurements revealed that all the complexes are non-electrolytes in nature. The infrared (IR) spectral studies indicates a tridentate behaviour for the ligand and binds to the transition metal ions through the azomethine nitrogen (>C=N) and caboxalato oxygen of cephalexin moiety. The electronic spectral results supported the predicted coordination geometry of the complexes. The in vitro antimicrobial activities of the compounds were assayed against some pathogens such as Gram-positive bacteria; Staphylococcus aureus and Bacillus subtilis; Gram-negative bacteria; Salmonella typhi and Escherichia coli and fungal strain; Aspergillus fumigatus. The assay revealed a good to excellent activity for the ligand and their complexes on all the tested microbes except in Aspergillus fumigatus with reference to the standard drugs (cephalexin and ketoconazole).***

Keywords: Schiff base ligand, Transition metals complexes, antimicrobial, Spectroscopic analysis,

I. INTRODUCTION

The antibiotic cephalexin (ceph) is first generation cephalosporin and has good activity against Gram-positive bacteria and relatively modest activity against Gram-negative microorganisms (Grady *et al*., 1997 and Karchmer, 1995).The structure of cephalexin metal complexes showing the involvement of α-amino group of acyl side chain in metal complexation was proposed by Anacona and Rodriguez (2004). The acyl side chain involved in metal chelation resulted in the antagonistic behavior of the cephalosporin. 2-aminopyridine (2-ampy) is one of the isomers of aminopyridines and it has been used for the synthesis of pharmaceutical agents, it is a colourless solid used in the production of piroxicam, which is used as a non-steroidal anti-inflammatory drug (NSAID) to relieve the symptoms of

ISSN: 2394-4404

painful, inflammatory conditions like arthritis (Brayfield, 2014). It has been used also as an intermediate for the synthesis of pharmaceutical agents such as sulfapyridine.

Schiff bases containing azomethine or imine (–C=N) unit is the condensation product of primary amines with carbonyl compounds and have been reported by Hugo Schiff (Schiff, 1864). Schiff base ligands with aldehydes are formed more readily than with ketones. This is because aldehydes react faster than ketones in condensation reactions and also reaction centres of aldehydes are less hindered than that of ketones as reported by [Adams *et al*., 2013; Hussain *et al*., 2015 ;Grace, 2015].

Transition metal complexes containing heterocyclic compounds have been of great interest in terms of structural chemistry, catalysis and biological functions (Millar *et al*., 1998). The field has undergone rapid growth due to the synthesis of multidentate ligands from heterocyclic compounds and the complexes of such ligands are formed with metal ions (Volpato *et al*.,2010 ; Zahan *et al.*, 2015). This paper reports the synthesis of a Schiff base ligand derived from cephalexin and 2-aminopyridine with its transition metal(II) complexes of Mn(II), Co(II) and Cu(II), with their physicochemical evaluations and antimicrobial activities .

II. MATERIALS AND METHODS

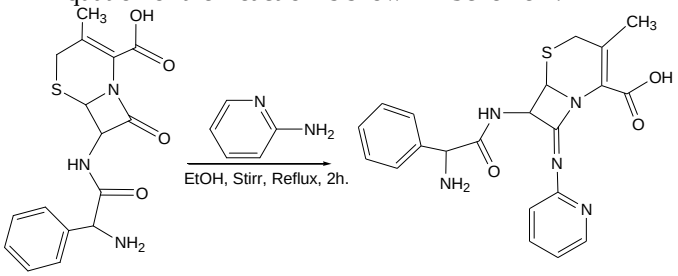
All chemicals were of analytical grade and were used without further purification. Cephalexin(Sigma Aldrich) and 2-aminopyridine (Sigma Aldrich) were obtained through Bristol Scientific Company, Lagos, Nigeria. Metal(II) salts used were: cobalt(II)chloride hexahydrate ($CoCl_2.6H_2O$), copper(II)chloride dihydrate ($CuCl_2.2H_2O$), (Fluka) except for manganese acetate (tetrahydrate)($Mn(OCOO)_2.4H_2O$, (Aldrich). Solvents used for synthesis and other characterizations such as solubility were of absolute purity which includes: ethanol, methanol, chloroform, n-hexane, benzene and acetone, dichloromethane (DCM), dimethylsulphoxide (DMSO) dimethylformamide (DMF), all from (Sigma Aldrich), distilled water.

The FT-IR spectral characterization of the compounds were recorded in the range of 400-4000 cm^{-1} as KBr disc on SHIMADZU Corporation, FTIR-8400S Spectrophotometer. For electronic Spectra, the instrument used was SHIMADZU - Probe UV-1800 UV-Vis Spectrophotometer in the range of 200-800 nm. For the ^{1}HNMR spectrum of the ligand, the instrument used was 5mm PABB –BB Spectrophotometer, which was measured in MeOD at 25^{o}C in the range of chemical shift of 0 - 10 ppm using TMS as standard at 400MHz.

SYNTHESIS OF THE SCHIFF BASE LIGAND (HL)

The Schiff base ligand (HL)was synthesized according to literature procedures (Imran, *et al*., (2007), Ndahi, *et al*., (2012), Taghreed, *et al*., (2014), Anacona, *et al*.(2016),by mixing cephalexin (0.34738g, 1mmol) with equimolar amount of 2-aminopyridine (0.09412g, 1mmol) dissolved in hot ethanol (25ml). The mixture was refluxed for 2 hours while progress of reaction was monitored by thin layer chromatography (TLC). On completion of the reaction, orange precipitate formed was allowed to cool, filtered and washed with ethanol followed by diethyl ether. The orange product was dried over calcium chloride ($CaCl_2$) in a desiccator.

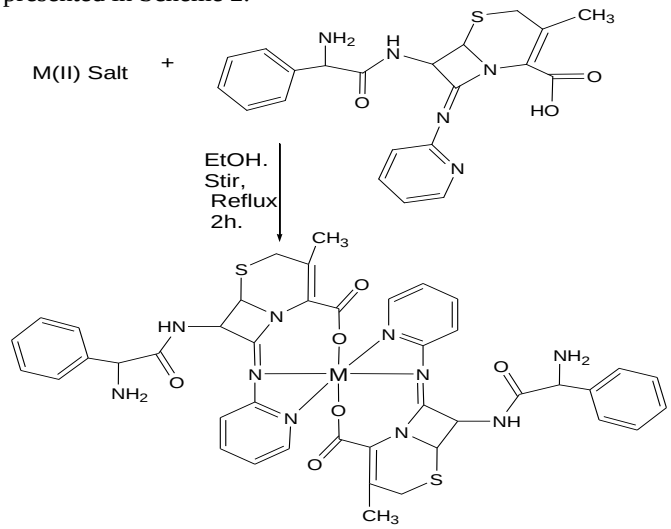
Equation of the Reaction is shown in Scheme 1.



Scheme 1: Schiff base derived from cephalexin antibiotic with 2-aminopyridine

PREPARATION OF THE METAL COMPLEXES

The transition metal complexes were prepared according to some modified literature procedures [Taghreed *et al*., (2014), Anacona *et al*., (2016)]. The Mn(II), Co(II) and Cu(II), complexes were prepared by dissolving 2mmol of the ligand and 1mmol of the corresponding metal ions in 1:2(M:L) mole ratio respectively. The reaction mixture was refluxed and continuously stirred using magnetic stirrer for 2hrs. The progress of reaction was each time monitored by thin layer chromatography (TLC). It was then allowed to cool at room temperature. The light brown or light green products formed were filtered and washed with ethanol, followed by diethyl ether and dried in a desiccator over $CaCl_2$.The reaction is presented in Scheme 2.



Where M = Mn(II), Co(II) and Cu(II)

Scheme 2: Synthesis of the metal(II) complexes

THE *IN VITRO* ANTIMICROBIAL ACTIVITY ASSAY

The results of the preliminary *in vitro* antimicrobial activity of the Schiff base ligand and its metal(II) complexes at concentrations of 30 and 20 µg/ml were used to determine the activity of the complexes and the ligand which were evaluated by Disc diffusion method as reported by Imran *et al*., (2007) and Al-Bayati*et al*., (2010) and adopted by Ndahi

et al. (2012); Wazari *et al.* (2013) on some clinical pathogens such as *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhi*, *Escherichia coli* and *Aspergillus fumigatus.* The inhibition zone was recorded in millimetre.

III. RESULTS AND DISCUSION

RESULTS

Compound	Colour	Conductivity ($Scm^2 mol^{-1}$)	M.P (^0C)	Yield (%)	Metal % Found (cacld)
HL(Ceph-2-ampy)	Orange	0.027	153-154	66	
[Mn(Ceph-ampy)$_2$]	Light brown	0.179	179-180	63	6.20(6.14)
[Co(Ceph-ampy)$_2$]	Light brown	0.122	193-195	66	6.50(6.25)
[Cu(Ceph-ampy)$_2$]	Light green	0.016	146-148	66	6.77(6.0)

Key: (Ceph-ampy) = Cephalexin-2-aminopyridine Ligand

Table 1: Some physical properties of the Schiff base and its metal(II) complexes

	Infrared Spectra of the ligand and its metal(II) Complexes(cm^{-1})						Electronic Spectra of the ligand and its metal(II) Complexes		
Compounds	**V(NH)**	**ν(OH)**	**ν(C=N)**	**ν(C-N)**	**ν(M-N)**	**ν(M-O)**	**λ_{max}(nm)**	**Wave number (cm^{-1})**	**Band Assignment**
Ceph-2-ampy (HL)	3461b	3265b	1661sh	1384s	-	-	229 275	43668 36363	$\pi\rightarrow\pi^*$ $n\rightarrow\pi^*$
[Mn(cehp-2-ampy)$_2$]	3390sh	2937sh	1640sh	1423sh	599sh	550sh	287 234	34843	$\pi\rightarrow\pi^*$
[Co(ceph-2-ampy)$_2$]	3356sh	2970sh	1668sh	1415sh	627sh	678w	294 234	34013	$\pi\rightarrow\pi^*$
[Cu(ceph-2-ampy)$_2$]	3445sh	2933sh	1622m	1384m	789sh	600sh	292 232	34247	$\pi\rightarrow\pi^*$

Table 2: Infrared spectra and Electronic Absorption Spectra of the ligand and its metal(II) complexes

Compound	**Conc. (µg/ml)**	***S. aureus***	***B. subtilis***	***S. typhi***	***E. coli***	***A,Fumigatus***
HL	30	32.33±0.33	30.66±0.33	20.00±0.0	17.66±0.33	R
	20	28.00±0.00	25.33±0.33	9.66±0.33	14.33±0.00	R
[Mn(HL)$_2$]	30	21.66±0.33	10.00±0.00	10.00±0.00	22.66±0.33	R
	20	10.33±0.33	10.00±0.00	10.00±0.00	200.33±0.33	R
[Co(HL)$_2$]	30	40.00±0.00	40.00±0.00	30.00±0.00	25.00±0.00	R
	20	9.66±0.33	30.00±0.00	10.00±0.33	20.33±0.33	R
[Cu(HL)$_2$]	30	31.66±0.33	29.33±0.00	23.00±0.33	26.66±0.66	R
	20	27.00±0.33	26.66±0.33	20.33±0.33	23.33±0.33	R
Cephalexin	30	26.00±0.00	20.00±0.00	23.00±0.00	20.33±0.33	R
Ketoconazole	30	-	-	-	-	31.33±0.33

Table 3: Antimicrobial Activities of the Schiff base Ligand HL and its Metal(II) Complexes

IV. DISCUSSION

The reaction between cephalexin with 2-aminopyridine in a molar ratio of 1:1(ceph:2-ampy), produced an orange solid of the Schiff base (HL)with a percentage yield of 66 %. The interaction of the Schiff base ligand and some metal(II) ions in ethanol in 1:2 molar ratio (M:L) gave the metal(II) complexes of the respective ions having various colours with a percentage yield ranging between 63-66%(Table1). The metal complexes formed are non-hygroscopic solids. The colours of the synthesized complexes can be attributed to d-d transitions of the metal ions (Lee, 2009). This is characteristic of transition metal complexes (Cotton, 1999) because of partially filled d-orbitals.

The ligand melted in the range of 153-154^{o}C. The melting points of the metal(II)complexes were recorded in the range of 146-195^{o}C(Table1). The high melting points of the Schiff base ligand and the metal(II) complexes indicated that they are stable compounds and not easily decomposed as reported by Ahmed and Akhtar, (1983).The higher melting points in the Mn(II) and Co(II)complexes compared to the ligand also suggest the chelating effect of the ligand(Antonio *et al*., 1984, Ndahi *et al.*, 2012). The percentages of each metal in the complexes are in agreement with the theoretical values. The solubility data indicated that the complexes are soluble in distilled water and some common organic solvents such as methanol and ethanol but slightly soluble or insoluble in n-hexane and acetone. They were however, very soluble in coordinating solvents such as DMF and DMSO .The low molar conductance values of the complexes revealed their non-electrolytic nature (Hultin, 2002).

INFRARED SPECTRA

In order to study the binding mode of the Schiff base to the metal complexes, the IR spectrum of the free ligand was compared with the IR spectra of the complexes (Table 2).The Schiff base (HL), shows its characteristic absorption bands in the 3461, 1661 and 1384 cm^{-1} regions, assignable to υ(O-H), υ(C=N) and υ(C-N) vibrations respectively. The band at 1661 cm^{-1}due to the azomethine functional group of the Schiff base underwent a shift to lower frequency in the Mn(II)(1640 cm^{-1}) and Cu(II) (1622cm^{-1}) complexes except the [Co(ceph-2-ampy)$_2$] which showed a band at 1668 cm^{-1}. This observation is similar to the literature reported by Byeong-Goo *et al.*, (1996), Aliyu and Sani,(2012), which indicates coordination of azomethine nitrogen to the metal ions. The nature of the metal-ligand bonding is confirmed by the appearance of the newly formed bands at 599- 789cm^{-1} and 550-678 cm^{-1} which are not present in the free Schiff base are assigned to υ(M-N) and υ(M-O) respectively. These observations agree with similar reports in the literature (Alaa,2015).

ELECTRONIC SPECTRA

The electronic spectral data of the ligand and the complexes were recorded in methanol in the wavelength range of 200-800nm and the results are presented in Table 2. The spectra of the compounds have been assigned by comparing the observed values with the previous works on similar systems (Tagreed *et al.*, 2014). The absorption spectra of the Schiff base exhibited two bands at 229 nm and 275 nm assigned to $\pi\rightarrow\pi^*$ and $n\rightarrow\pi^*$ transitions of the ligand. The inner ligand transitions such as $\pi\rightarrow\pi^*$ and $n\rightarrow\pi^*$ are expected due to the presence of C=N, C=O and C=C groups in the ligand

structure(Lever,1968) In the complexes, these bands were shifted to lower frequency of 232-294 nm which were also assigned to $\pi \rightarrow \pi^*$ or $n \rightarrow \pi^*$ transitions which indicates coordination. This is in agreement with the literatures reported by Somez *et al.*,(2004) and Anacona *et al*., (2014). This observation may also be due to polarization within the C=N- caused by metal-ligand electron interaction.

^{1}HNMR SPECTRUM

The ^{1}H NMR spectral data of the free Schiff base ligand was recorded in MeOD against tetramethylsilane(TMS) as internal reference. The aromatic protons, which appeared as a multiplet were observed at 7.2 – 8.3 ppm. The signals due to the methyl protons were observed at 1.5 ppm. The NH_2 protons were seen at 4.2 ppm. The chemical shifts obtained were similar to those of Schiff base ligands reported by Tagreed *et al.*,(2014) and Anacona *et al*., (2014).

ANTIMICROBIAL ASSAY

The antimicrobial activity of the Schiff base shows promising activities against all the bacteria used except *E. coli*. The complexes of cobalt(II) and copper(II) possess pronounced bactericidal activity against *S. aureus, S.typhi, B.subtilis* and *E. coli* in comparison with the manganese(II) complex which is active only against *E. coli and S. aureus* compared to that of the reference drug cephalexin. The fungi (*A.Fumigatus*) showed resistance at all the concentrations used.

CONCLUSION

The results of the analysis have revealed that cephalexin antibiotic has condensed with the 2-aminopyridine which afforded Schiff base ligand (HL). Metal analysis, IR spectra and UV/visible electronic transitions were used to support the proposed structures. The ligand and the metal complexes exhibited moderate to excellent activity towards all the mentioned bacterial strains. On the basis of the results obtained, the proposed structure of the ligand and complexes are as shown in Schemes 1&2 respectively.

ACKNOWLEDGEMENTS

The authors wish to thank all the laboratory staff of Chemistry Department, University of Maiduguri, for their kind assistance. The Department of Veterinary Medicine, University of Maiduguri is also acknowledged for their support in the antimicrobial work and also, the UniversitiTeknologi Malaysia 81310 Johor Bahru – Instrument Room, Department of Chemistry, Faculty of Science, Malaysia, for their support in Infrared, Electronic spectral and ^{1}HNMR.

REFERENCES

[1] Antonio, B. Francesco, D.and Giovanni, M .P. I (1984). Chelate effect and cooperativity effect in metal-ligand and macromolecule-ligand equilibria. I. Chemical potential changes and cooperativity-chelation parameters. Inorganica Chimica Acta,91, (3), 195-201

[2] Adams, M., Li, Y., Khot, H., De Kock, C., Smith, P. J., L and, K.,Chibale, K. and Smith, G. S.(2013). The synthesis and antiparasitic activity of aryl- and ferrocenyl-derived thiosemicarbazoneruthenium(II)-arene complexes.Dalton Transactions,42, 4677–4685.

[3] Ahmed, A. and Aktar, F. (1986). Cu(II) and Ni(II) complexes with a tetradentate Schiff base derived from 2-hydroxy-1-napthaldehyde and ethylenediamine. Indian Journal of Chemistry, 20A: 737-758.

[4] Alaa, E. A, Mamdouh, S. M. and Nessma M. N (2015).Coordination chemistry and biological activity of different types of antibiotics, Journal of Chemical and Pharmaceutical Research, 7(7):64-90

[5] Al-Bayati, R. I. H., F .R and Ahmed, A. H .A. (2010). Synthesis, Spectroscopic and Antimicrobial studies of Transition Metal Complexes of N-amino Quinolone Derivatives. 14thInternational Electronic Conference on Synthetic Organic Chemistry (ELSOC-14): 1-30.

[6] Aliyu, H.N. and Sani,U. (2012). Synthesis, characterization and biological activity of manganese(II), iron(II), cobalt(II), nickel(II) and copper(II) Schiff base complexes against multi drug resistant bacterial and fungal pathogens. International Research Journal of Pharmacy and Pharmacology (ISSN 2251-0176). 2(2), 040-044,

[7] Anacona, J. R., Calvo, G. and Camus, J. (2016). Tetradentate Hydrazones Metal Complexes derived from Cefazolin and 2,6−diacetylpyridine Hydrazide: Synthesis, Characterization, and Antibacterial activity. International Journal of Chemistry, Monatsh. Fur Chemie,147. (18): 725-733

[8] Anacona, J. R., Rodriguez, J. L. and Camus, J. (2014). Synthesis, Characterization and Antibacterial activity of a Schiff Base Derived from Cephalexin and Sulphathiazole and its Transition Metal Complexes. Spectrochimica Acta, Part A,129: 96-102.

[9] Brayfield, A. (2014) "Piroxicam". Martindale: The Complete Drug Reference. Pharmaceutical Press, London, UK.

[10] Byeong-Goo J, Chae -Dyong R, Hee-Name C, Ki-Hyung C,Yohng-Kook. Synthesis and characterization of schiff base derived from 2-hydroxyl-1-1 naphthaldehyde and aliphatic diamines bull. Korean Chem. Soc. 1996;17(8):687-693.

[11] Cotton, F.A., Wilkinson, G., Murillo, C.A, and Bochmann, M. (1999). Advanced Inorganic Chemistry. Sixth Edition. John Wiley and Sons, Inc. 3,590-872.

[12] Grace, E .I. (2015). Synthesis, characterization and antimicrobial studies of Mn(II), Co(II), and Zn(II) Schiff base complexes derived from glycine and 2-hydroxy-1-napthaldehyde. International Journal of Innovation and Scientific Research, 18 (1), 1-3.

[13] Grady, O. F, Finch, R. G, Lambert, H.P. and Greenwood,D. (1997). Antibiotic and Chemotherapy, Anti-infective agents and their use in therapy, 7th edition, Churchill Livingstone, New York, .202-15.
[14] Hultin, P.G.(2002). A Guide to Solvents and Reagents in Introductory Organic Chemistry for students, 2, 222-223.
[15] Hussain, Z., Yousif, E. and Taher, B. (2015) Schiff bases derivative, Schiff bases containing sulfamethoxazole nucleus, Lambert academic publishing, Germany.(6) 20.
[16] Imran, M., Iqbal, J., Iqbal, S. and Ijaz, N.(2007). In vitro Antibacterial Studies of Ciprofloxacin-imines and their Complexes with Cu(II),Ni(II),Co(II), and Zn(II). Turkey. Journal of Biology, (31): 67–72.
[17] Lee, J. D. (2009). Concise Inorganic Chemistry, fifth Ed. Wiley India (ed) (P.) Ltd. 4435/7, Ansari Road, Daryaganj, New Delhi.,751, 799, 815, 833.
[18] Millar M.C., Bastow K.F., Stineman C.N., Vance J.R., Song S.C., West D.X.and Hall I.H. (1998); Anticancer Research, Archives Pharmaceutical Medicinal Chemistry. 18, 4131
[19] Ndahi, N. P., Nasiru Y. P, and Sandabe, U. K.(2012). Synthesis, Characterization and Antibacterial Studies of Some Schiff Base Complexes of Co(II), Ni(II) and Zn(II). Asian Journal of Biochemical and Pharmaceutical Research,1 (2), 2231-2560.
[20] Schiff, H. (1864). Communications from the University laboratory in Pisa: a new range of organic bases. Analytical Chemistry, 131: 118-119.
[21] Somez, M., Lovent, A.and Skerchi,M. (2004).Synthesis and characterization of some transition metal complexes of a bidentate Schiff base and their antifungal and antimicrobial studies. Russian Journal of Coordination Chemistry. 30 655-659
[22] Taghreed, H., Al-Noor., Ahmed, T., AL-eboori, and Manhel, R. (2014). Preparation, Characterization and Antimicrobial activities of Fe(II),Co(II),Ni(II),Cu(II),and Zn(II) Mixed Ligand Complexes of a Schiff base derived from Cephalexin drug and 4 (dimethylamino) benzaldehyde with Nicotinamide. Journal of Advances in Physics Theories and Applicatoins. (18),1-10.
[23] Karchmer, A.W. (1995). Cephalosporins, In Mandell, Douglas and Bennett". Principles and
[24] Practice of Infectious Diseases, 4th edition, Churchill Livingstone, NewYork.247-263.
[25] Volpato, G.; C. Rodrigues, R. and Fernandez-Lafuente, R. (2010). Use of Enzymes in the Production of Semi-Synthetic Penicillins and Cephalosporins: Drawbacks and Perspectives, Journal of Current Medicinal Chemistry, 17, (32): 3855-73.
[26] Waziri, I., Ndahi, N. P. and Paul, B. B. (2013). Synthesis, Physicochemical and Antimicribial Studies of Co(II), Zn(II) and Fe(III) Mixed antibiotics Metal Complexes, Journal of Chemical and Pharmaceutical Research, 5(9): 84-89.
[27] Zahan, M. K. E., Islam,M. S.and Bashar, M. A. (2015). Synthesis,Characterization and antimicrobial activity of Some Metal Complexes of Mn(II), Fe(III) Co(II), Ni(II), Cu(II) and Sb(III) Containing Bidentate Schiff base of SMDTC. Russian Journal of general chemistry 85: 667-672.